Oligo Design Across the Mouse Genome

Ben Sowers
Senior Scientist
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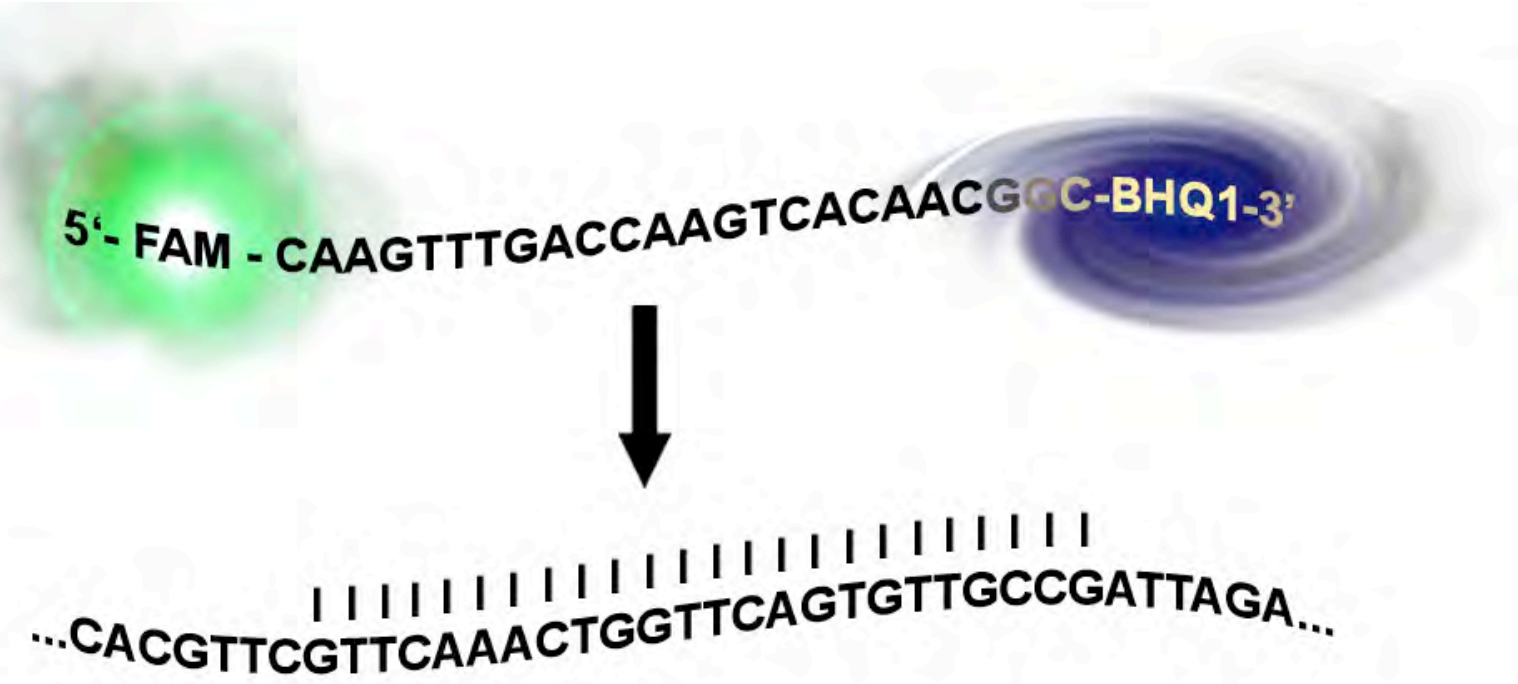
81 Digital Dr, Novato, CA 94949-5750
1.415.883.8400 • 1.415.883.8488 (fax)
www.biosearchtech.com

Fluorescence-Quenched Probes



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"the combination of the hybridization-specific probes and PCR amplification provided combined sensitivity, speed, specificity, and simplicity."

-Morrison, et al.

Morrison L. E., et al. (1989) Solution-phase detection of polynucleotides using interacting fluorescent labels and competitive hybridization. Analytical biochemistry 183(2): 231-244.

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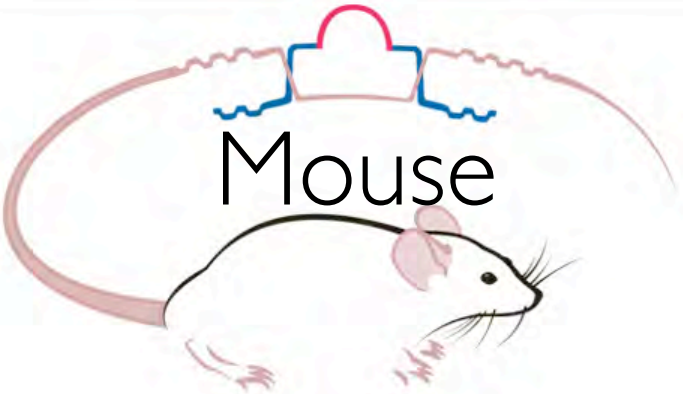
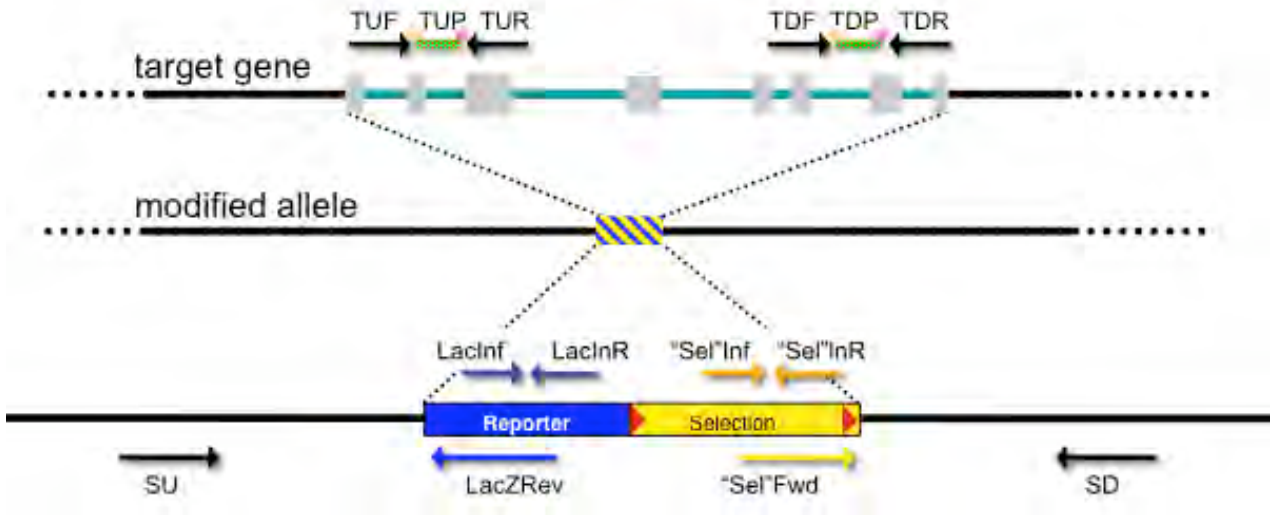
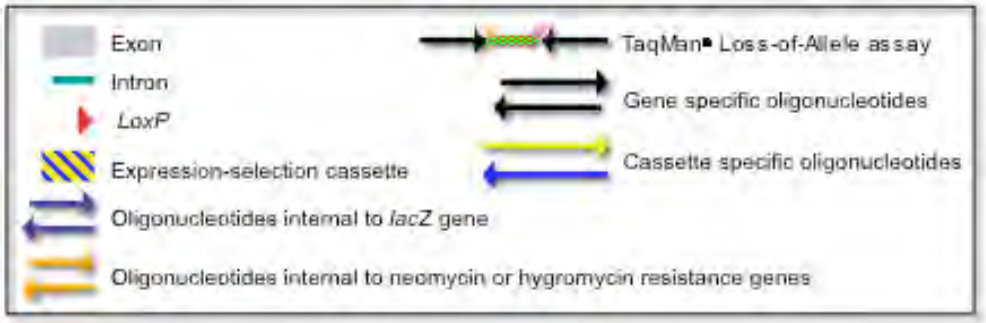
Knockout Mouse Project



VelociGene's KOMP Allele Genotyping Strategies





Valenzuela, D. M., et al. (2003). High-throughput engineering of the mouse genome coupled with high-resolution expression analysis. Nat Biotechnol 21, 652-659.

RealTimeDesign™

www.qpcrdesign.com

High Throughput:

Ten different assays designed simultaneously.

Adjustable Parameters

Custom values to make default for future.

Design Archives:

History stores 100 different design sessions.

Free to use,
entirely through the web.

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Oligo Design Considerations

- Unique identification of the target sequence

CATAATCGTTGCTATTAACCACTTTATGAAAATCTAACTGGACATGGTTTTTCACTACTTTTAGCAGGTGG

- Binding stability that is tuned to the temperature profile of the assay

- Signal presence of the amplifying target

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Sequence Designs to Date

86.5% of all assays designed using three default parameter sets.

The Most Restrictive parameters yield a 96.7% success rate.

The Less Restrictive parameters yield a 96.4% success rate.

The Least Restrictive parameters yield a 94.4% success rate.

Assays designed on a custom basis yield a 83.6% success rate.

> 5,000 assays synthesized.

> 4,500 have been tested.

~ 93% success rate for new designs.

~ 80% success rate for re-designs.

Difficult targets demand BHQplus™

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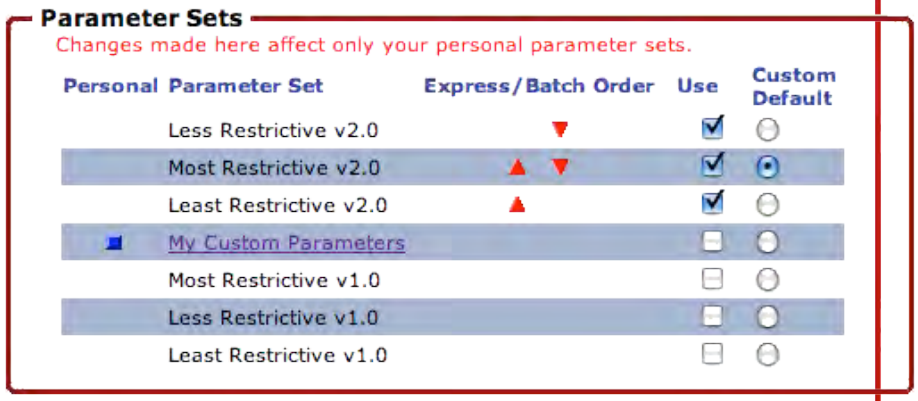
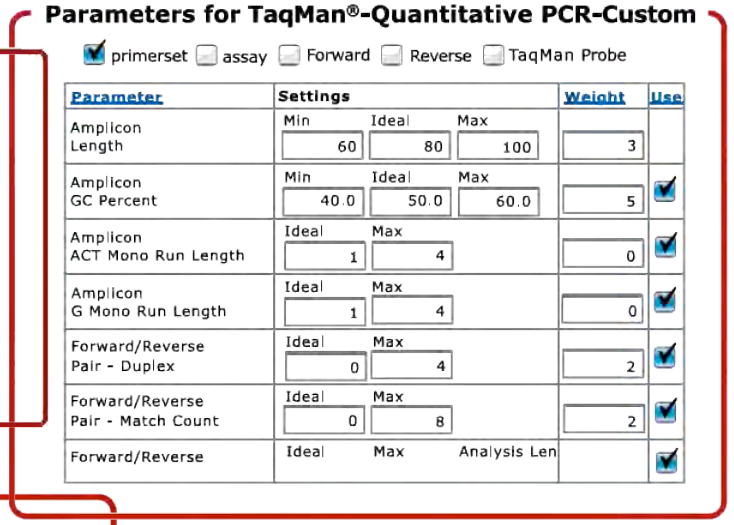
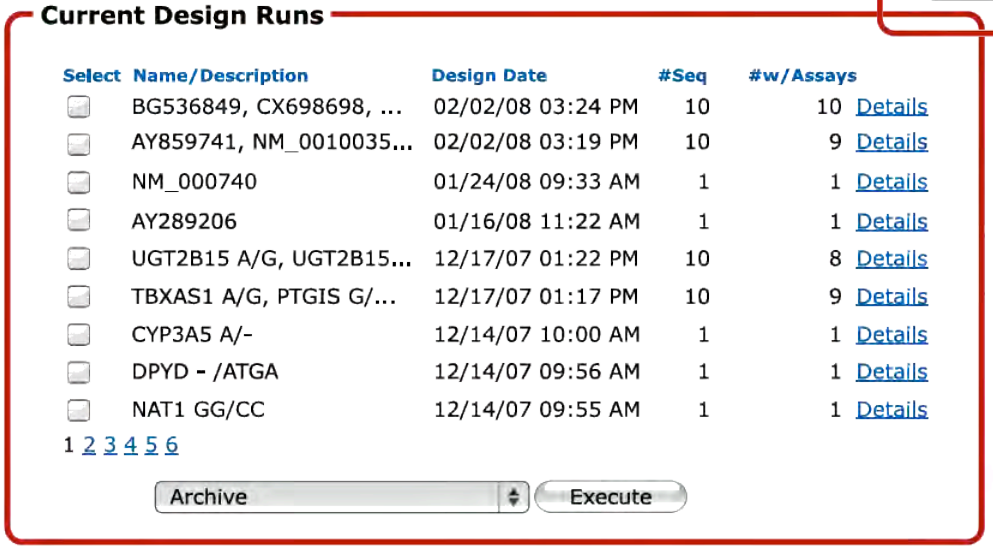
Design Archives & Custom Parameters







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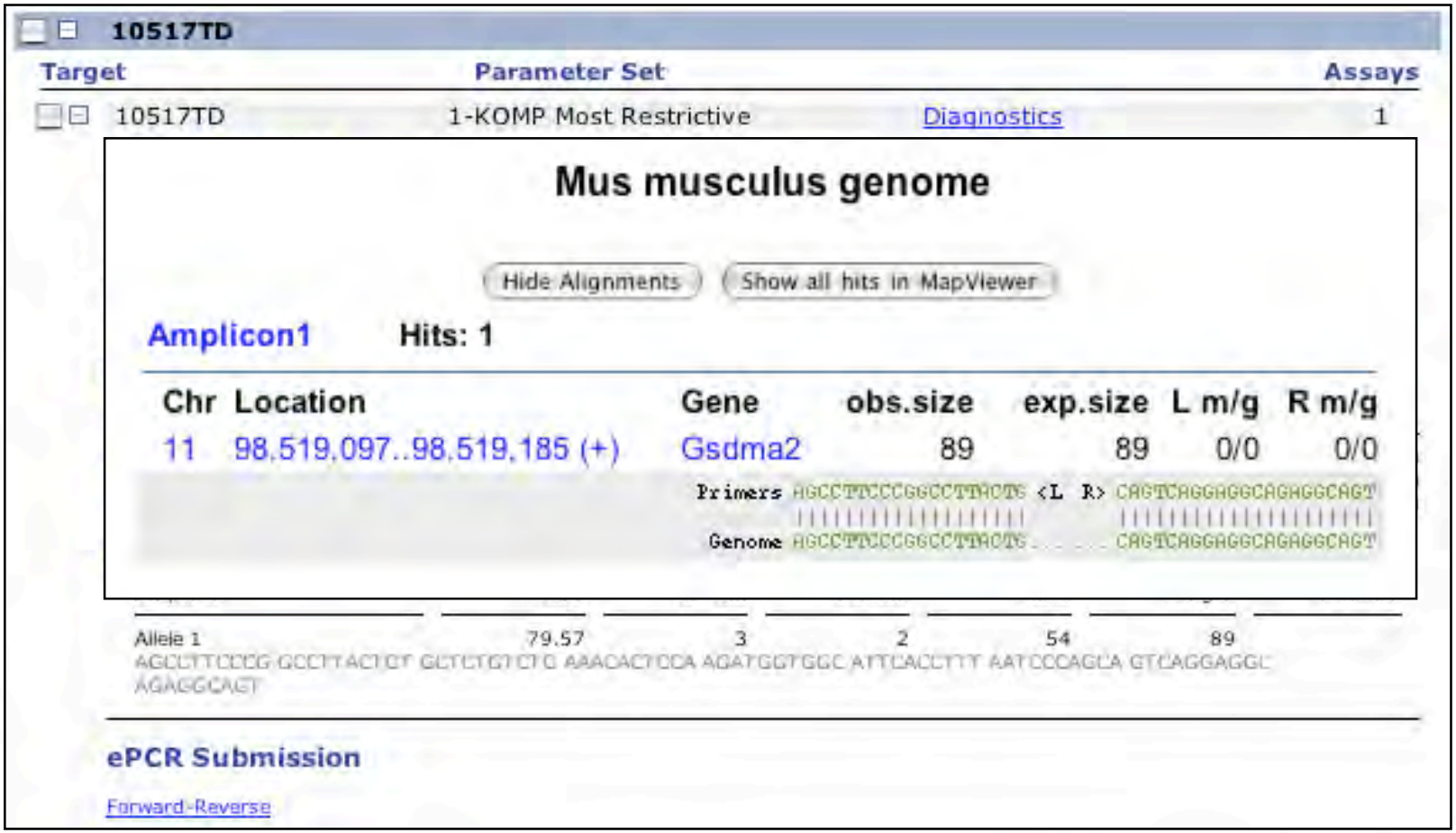
Assay Design - Gsdma2



Confirm assay specificity *in silico*

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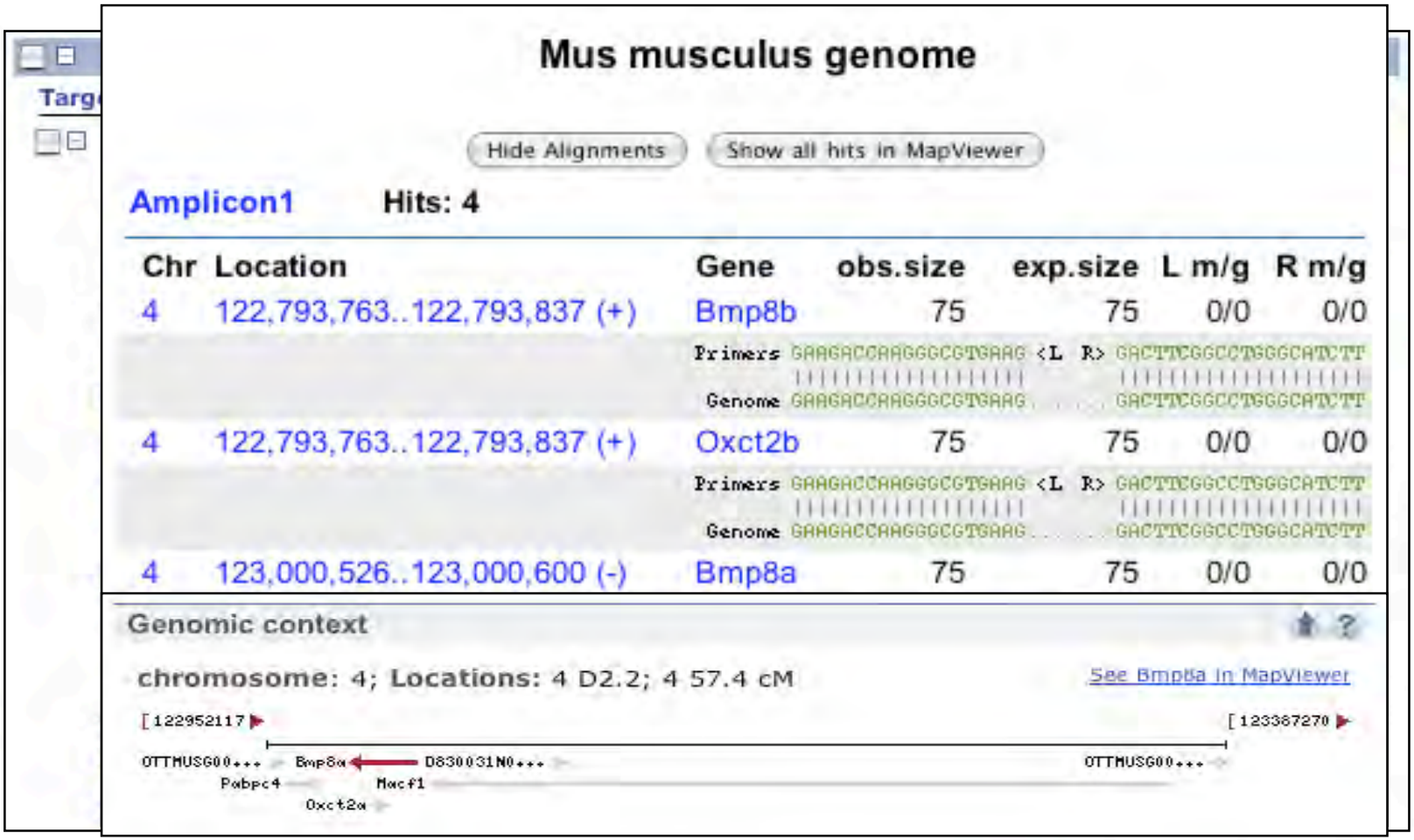
Assay Design - Bmp8a



Confirm assay specificity *in silico*

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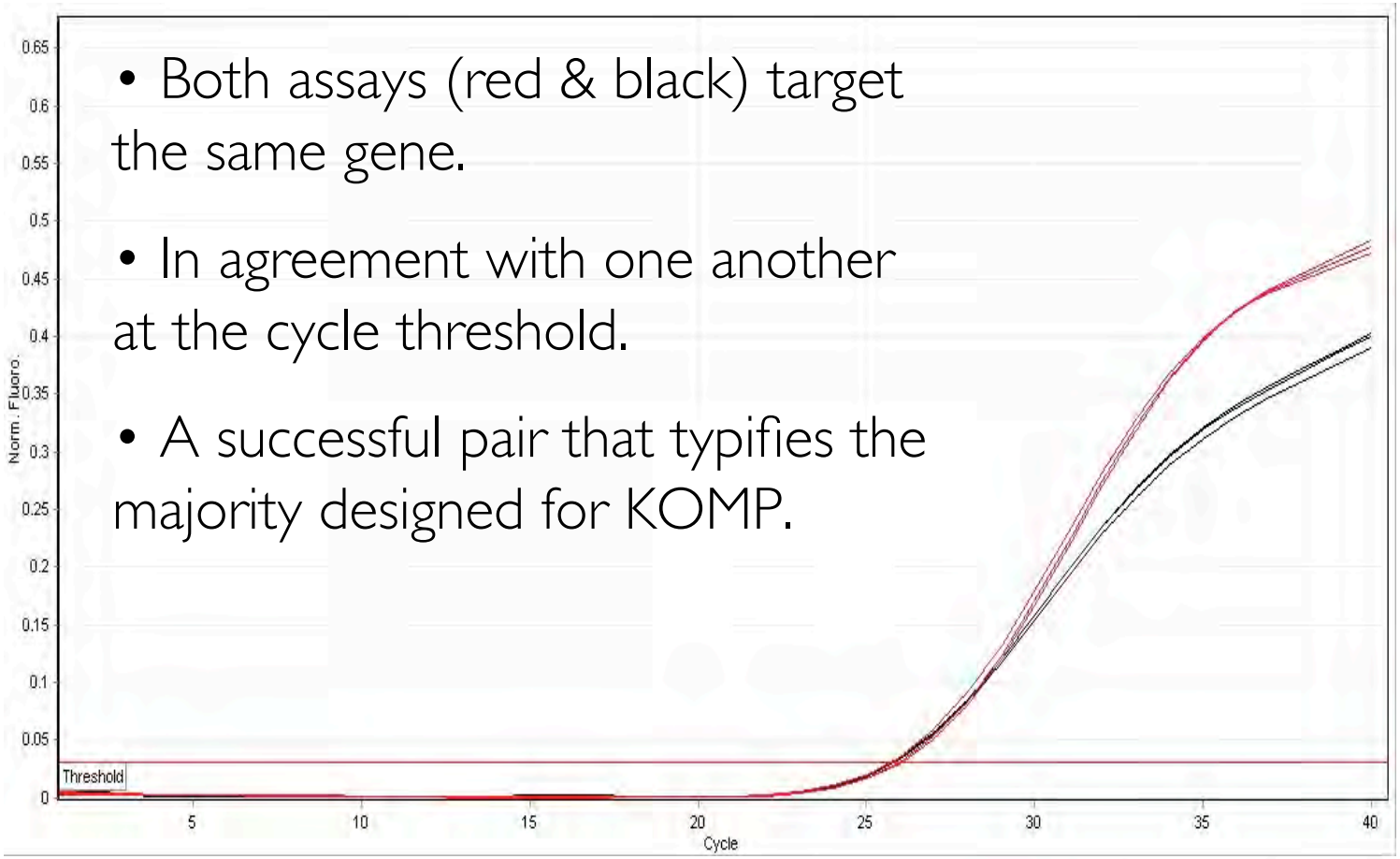
Amplification Performance - Gsdma2



- Both assays (red & black) target the same gene.
- In agreement with one another at the cycle threshold.
- A successful pair that typifies the majority designed for KOMP.

Confirm amplification *in vitro*

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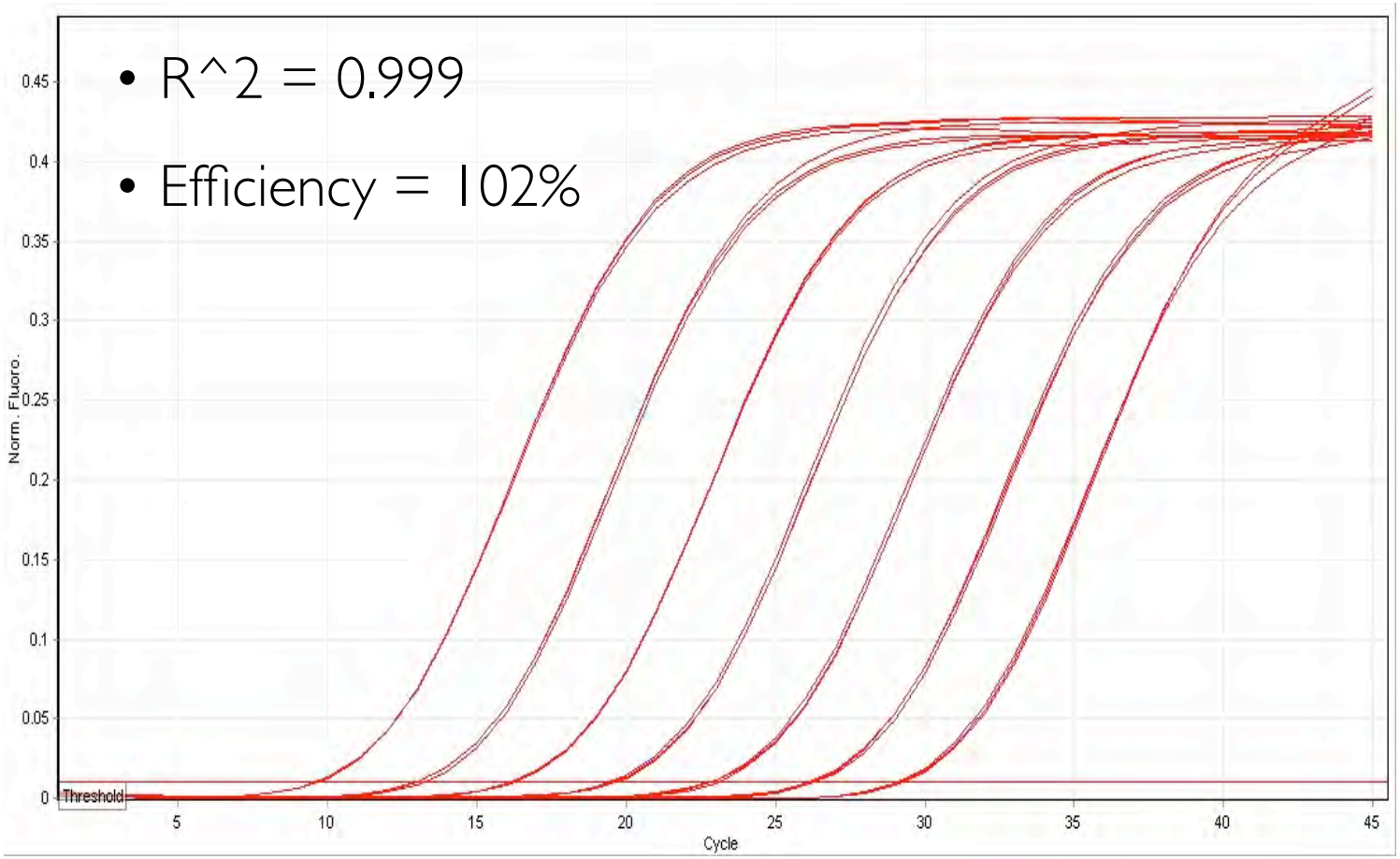
Amplification Performance - Gsdma2



Confirm amplification *in vitro*

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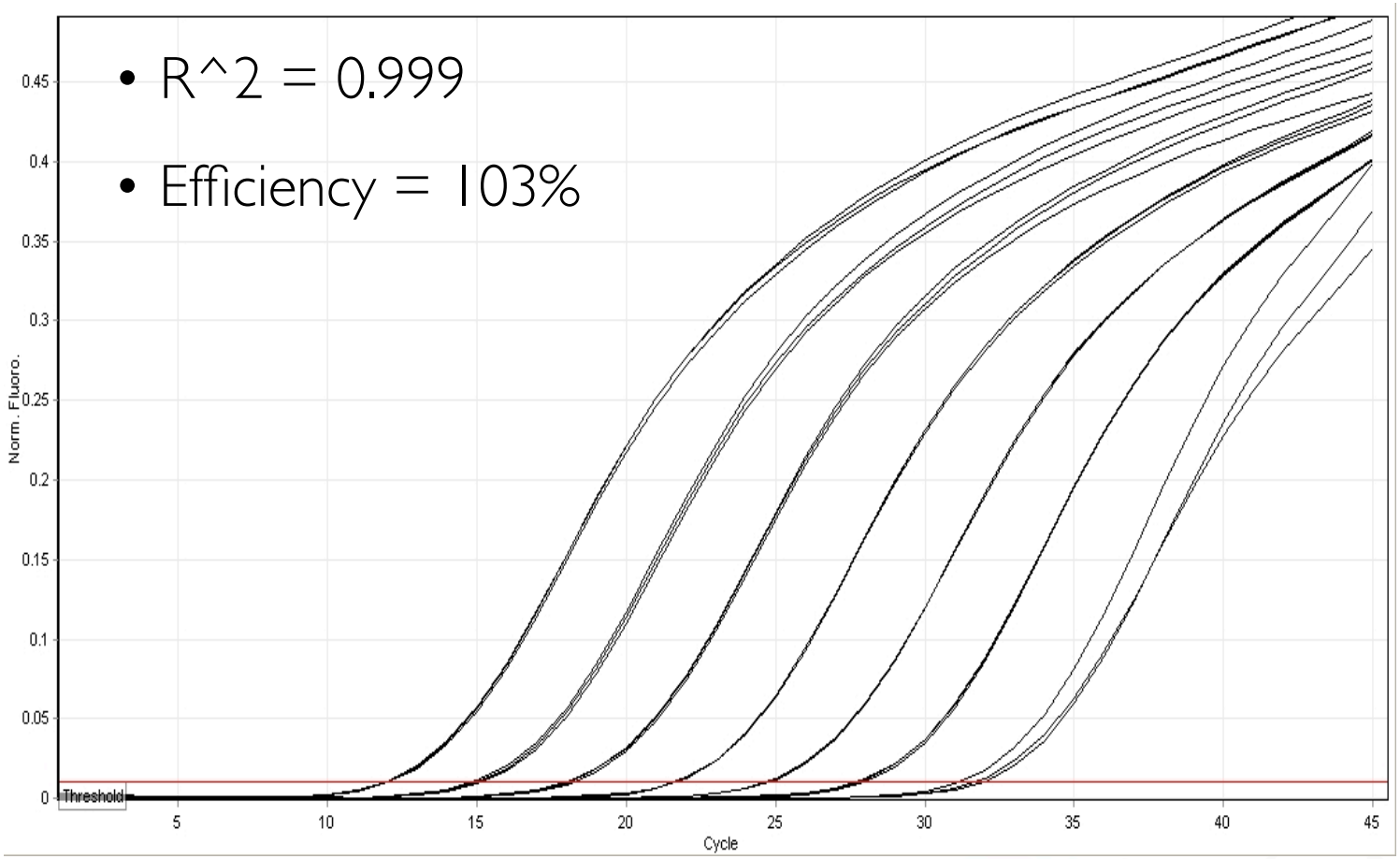
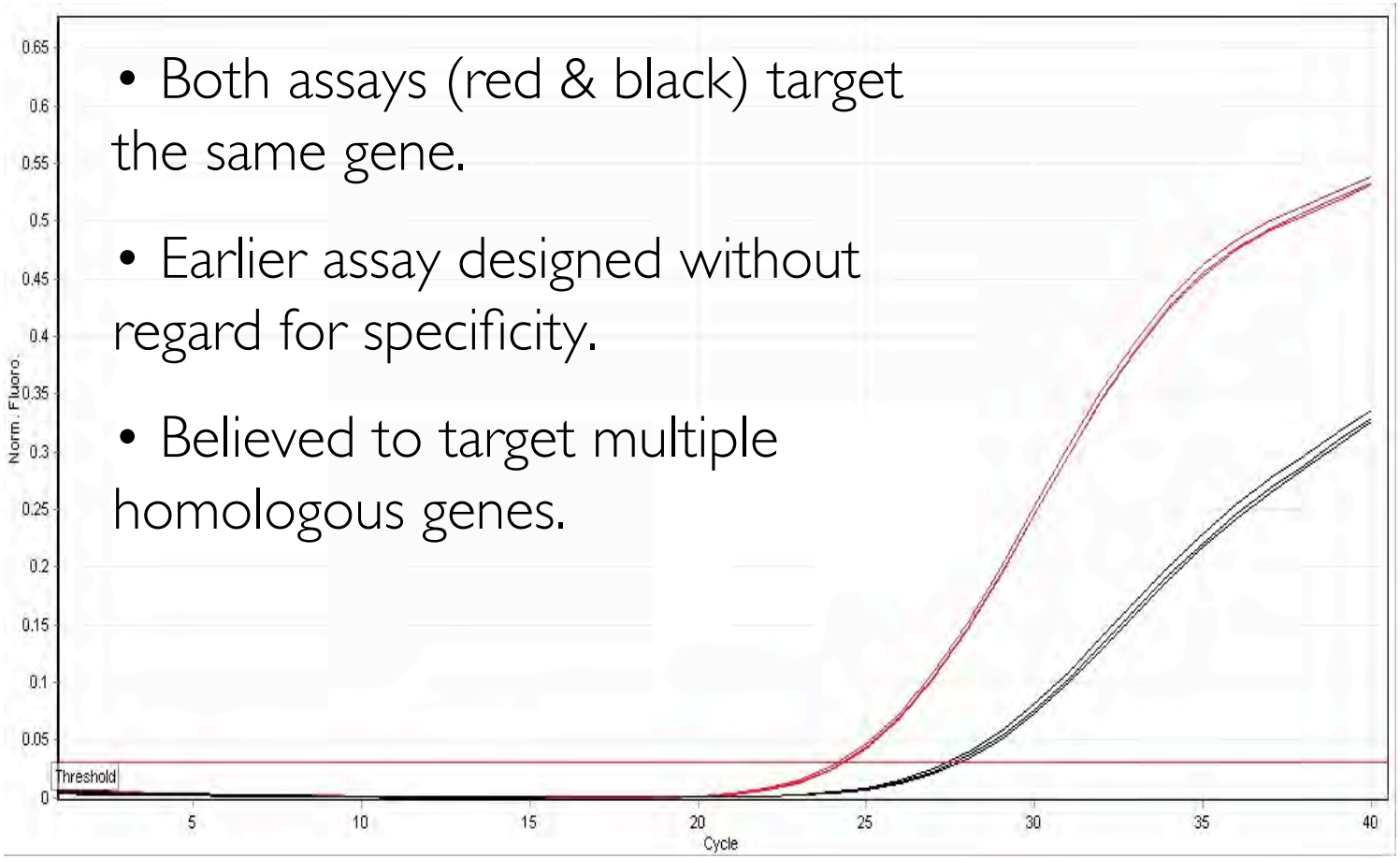
Amplification Performance - Gsdma2



Confirm amplification *in vitro*

BIOSEARCH TECHNOLOGIES

Amplification Performance - Bmp8a



- Both assays (red & black) target the same gene.
- Earlier assay designed without regard for specificity.
- Believed to target multiple homologous genes.

Confirm amplification *in vitro*

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ACKNOWLEDGEMENTS

Yingzi Xue, Rostislav Chernomorsky, David Frendeway and others in the Velocigene® division of Regeneron.

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